



In utero gene delivery to spinal cord motor neurons



Gene therapy is one of the most exciting approaches in combating various diseases. Its potential, however, has not been fully realized since the first human gene therapy trial began almost three decades ago. Gene therapy, simple and elegant in principle, is indeed extremely difficult. Of the many parameters critical to successful gene therapy, two particularly relevant factors are targeted delivery and expression of therapeutic genes into specific cell populations. Development of a gene therapy with high efficiency and low toxicity requires the right combination of a delivery route and a vector used. The central nervous system, including the spinal cord, is a particularly critical target which can be affected by a number of genetic diseases that start developing before birth. *In utero* gene therapy is a promising route for gene delivery which conveys distinct advantages over traditional gene therapy.

Fetal delivery of transgenes may prevent the pathology of early-onset diseases, induce tolerance against an expressed therapeutic protein and increase the likelihood of widespread transduction [1]. Lentiviral vectors can carry fairly large payloads and transduce both quiescent and dividing cell populations. Integration-deficient lentiviral vectors (IDLVs) offer additional safety advantages, becoming episomal molecules and minimizing the risk of insertional mutagenesis [2]. The manuscript by Sherif Ahmed et al. and his team in this issue describes the utilization of IDLVs for delivering genes to the spinal cord through intraspinal injection into fetal mice [3]. This work demonstrates highly efficient and persistent transgene expression in motor neurons within the mouse spinal cord.

Professor Yáñez-Muñoz and his team collaborated with Dr. Simon Waddington, an *in utero* delivery expert. They first identified the optimal fetal route of injection for gene delivery to the spinal cord using IDLVs carrying an enhanced green fluorescence protein (eGFP). Intraspinal, intracranial, or intravascular routes of injection were tested in E16 fetal mice. The intraspinal injection showed robust eGFP fluorescence along the entire spinal cord and in spinal ganglia, while the effects of intracranial and intravenous injections were restricted to the brain and the liver, respectively. The Yáñez-Muñoz team then optimized a vector type and specificity. IDLVs containing cytomegalovirus (CMV)-eGFP and pseudotyped with vesicular stomatitis virus G glycoprotein (VSV-G), Rabies, Mokola or Ross River virus envelope proteins were tested in comparison to self-complementary adeno associated viral vectors serotype 9 (scAAV9). Widespread eGFP fluorescence was confirmed in whole spinal cord and ganglia of animals injected intraspinally with IDLVs and scAAV9. To assess efficiency and cell-type specificity of

transgenic expression after *in utero* delivery of IDLVs and scAAV9, transduction in the major CNS cell types (motor neurons, neurons, astrocytes, oligodendrocytes and microglia) was evaluated histologically. Co-staining for markers of the relevant cell types revealed motor neurons to be most efficiently and broadly transduced with IDLVs. To assess the stability of transgenic expression following *in utero* intraspinal delivery, the Yáñez-Muñoz team harvested animals seven months post-birth. No difference with the P10 time-point was apparent; the pattern and cell-type distribution of eGFP expression were stable, with no obvious reduction in the frequency of eGFP+ cells over time.

The work by Professor Yáñez-Muñoz and his colleagues presents a promising tool not only in studies of CNS biology and disease but also in clinical translation of gene therapy. The selective, extensive and highly efficient transduction of choline acetyltransferase (ChAT)-positive motor neurons and dorsal root ganglia neurons creates huge opportunities for a number of novel approaches. The key features of this approach include the early time point at which genes can be introduced into the nervous system, the selectivity for ChAT-positive cells and the relatively low number of IDLV particles required. Given the importance of motor neurons in neuromuscular disease, these findings may also have therapeutic implications for diseases such as spinal muscular atrophy. For clinical applications, the *in utero* gene therapy is only possible after determination of a genetic disorder. This may not be difficult, as the current technology allows routine testing of genetic disorders. The study by the Yáñez-Muñoz team has laid another stepping stone for successful translations of the gene therapy concept to routine clinical applications.

References

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